

PRELIMINARY AMENDMENT

Continuation of U.S. Appln. No. 09/125,944

- (2) detecting a trace of digestion formed on the thin membrane by the action of protease.
2. (amended) A method for measuring protease which comprises the steps of:
- (1) contacting one of two substantially continuous slices of a biological sample with a thin membrane which comprises a protease substrate together with a hardening agent formed on a surface of a support;
- (2) detecting a trace of digestion formed on the thin membrane by the action of protease; and
- (3) comparing the trace of digestion with a histopathological preparation prepared from the other slice.
3. (amended) A method for measuring protease which comprises the steps of:
- (1) contacting one of two or more substantially continuous slices of a biological sample with a thin membrane which comprises a protease substrate together with a hardening agent formed on a surface of a support;
- (2) contacting the remaining slices with a thin membrane which comprises a protease substrate, a hardening agent, and a protease inhibitor formed on a surface of a support;
- (3) detecting traces of digestion formed on the thin membranes by the action of protease; and
- (4) comparing the trace of digestion on the thin membrane used in step (1) with the trace of digestion on the thin membrane used in step (2).
4. (amended) A method for measuring protease which comprises the steps of:

PRELIMINARY AMENDMENT  
Continuation of U.S. Appln. No. 09/125,944

- (1) contacting one of two or more substantially continuous slices of a biological sample with a thin membrane which comprises a protease substrate together with a hardening agent formed on a surface of a support;
- (2) contacting the remaining slices with a thin membrane which comprises a protease substrate different from the protease substrate present in the thin membrane used in step (1) together with a hardening agent formed on a surface of a support;
- (3) detecting traces of digestion formed on the thin membranes by the action of protease; and
- (4) comparing the trace of digestion on the thin membrane used in step (1) with the trace of digestion on the thin membrane used in step (2).
5. (amended) A method for measuring protease which comprises the steps of:
- (1) contacting a sample containing protease with a thin membrane which comprises at least the following two layers: layer (a) which contains a protease substrate, a hardening agent, and a protease inhibitor formed on a surface of a support, and layer (b) which contains a protease substrate and a hardening agent laminated on layer (a);
- (2) detecting traces of digestion formed on the thin membrane by the action of protease; and
- (3) comparing the trace of digestion on layer (a) with the trace of digestion on layer (b).
6. (amended) A method for measuring protease which comprises the steps of:
- (1) contacting a sample containing protease with a thin membrane which comprises at least the following two layers: layer (a) which contains a protease substrate together with a

PRELIMINARY AMENDMENT

Continuation of U.S. Appln. No. 09/125,944

hardening agent formed on a surface of a support, and layer (b) which contains a protease substrate different from the protease substrate present in layer (a) together with a hardening agent laminated on layer (a);

- (2) detecting traces of digestion formed on the thin membrane by the action of protease; and
- (3) comparing the trace of digestion on layer (a) with the trace of digestion on layer (b).

7. (amended) The method of claim 1 wherein the protease substrate is selected from the group consisting of collagen, gelatin, proteoglycan, fibronectin, laminin, elastin, and casein.

8. (amended) The method of claim 1 wherein the sample is a biological sample isolated or collected from a patient.

9. (amended) The method of claim 1 wherein the detecting by using a thin membrane containing one or more substances selected from the group consisting of metals, metal oxides, pigments and dyes and having a maximum transmission density of 0.01 or higher at a wavelength ranging from 400 nm to 700 nm.

10. (amended) The method of claim 1 wherein the protease is a matrix metalloproteinase.

11. (amended) A thin membrane for measuring protease which contains a protease substrate together with a hardening agent formed on a surface of a support.

12. (amended) The thin membrane of claim 11 which comprises at least the following two layers:

PRELIMINARY AMENDMENT  
Continuation of U.S. Appln. No. 09/125,944

layer (a) which comprises a protease substrate, a hardening agent and a protease inhibitor formed on a surface of a support, and layer (b) which contains a protease substrate together with a hardening agent laminated on layer (a).

13. (amended) The thin membrane of claim 11 which comprises at least the following two layers: layer (a) which comprises a protease substrate together with a hardening agent formed on a surface of a support, and layer (b) which comprises a protease substrate different from the protease substrate present in layer (a) together with a hardening agent laminated on layer (a).

14. (amended) The thin membrane of claim 11 which comprises one or more substances selected from the group consisting of metals, metal oxides, pigments and dyes and have a maximum transmission density of 0.01 or higher at a wavelength ranging from 400 nm to 700 nm.

15. (amended) The thin membrane of claim 11 wherein the support is a microscope slide or a polyethylene terephthalate film.

16. (amended) The thin membrane of claim 11 wherein an undercoat layer is present between the support and the thin membrane.

17. (amended) A method of diagnosing a disease involving protease which comprises the steps of:

(1) contacting a biological sample isolated or collected from a patient with a thin membrane which comprises a protease substrate together with a hardening agent formed on a surface of a support; and

PRELIMINARY AMENDMENT

Continuation of U.S. Appln. No. 09/125,944

(2) detecting the trace of digestion formed on the thin membrane by the action of  
protease.

18. (amended) The method of claim 17 wherein the disease is selected from the group  
consisting of cancer, rheumatic diseases, periodontal diseases and alveolar pyorrhea.

---

APPLIED FOR